Results and Discussion

Human olfactory threshold variation

Detection thresholds of 4 odorants: Isoamyl acetate (IAA), Isovaleric acid (IVA), L-Carvone (LCA) and Cineole (CIN), were measured in 443 individuals. A broad Gaussian distribution was seen for all odorants, spanning the entire concentration range (10^{-2}-10^{-6}M, Figure 1). The reproducibility of the threshold determination was indicated by a test-retest correlation ($R=0.73 \pm 0.03$, average of 4 odorants) in 82 randomly selected (Figure S1). Examination of other potential confounding factors revealed a noticeable difference between the thresholds of the two genders (Table 1 in the Supplementary data). Age, smoking habits and ethnic origin did not show an effect.

Genotype-phenotype association

We analyzed the underlying disrupting single-nucleotide polymorphisms (SNPs) of 43 OR segregating pseudogenes (Table 1 in the Supplementary Data) in 377 individuals for which genomic DNA was available. Based on what is generally known about specific hyposmia, Mendelian recessive inheritance was assumed and grouping homozygously intact and heterozygote individuals was performed. Comparing the thresholds of individuals carrying different OR genotypes revealed a strong association signal between the inactivating SNP in *OR11H7P* and sensitivity to the odorant Isovaleric acid (Figure 2). This association is explicitly revealed in the isovaleric acid threshold distribution (Figure 1B), whereby subjects homozygous for the disrupted allele are significantly depleted in the two highest olfactory sensitivity bins (specific hyperosmia). This observation is in line with the notion that subjects bearing no functional copy.

Interestingly, another association signal to IVA (however not as statistically significant) is with a SNP in the *OR4Q2P* gene which is situated ~225 kb downstream the OR11H7P gene within the same OR genomic cluster 14@19.5 [11] (Figure 3A). The genetic association observed at the *OR11H7P* locus can be an indirect association attributable to linkage disequilibrium to causative polymorphisms in other OR genes within the same genomic region (Figure 3A).

Figure 3B indicates that the predicted binding site (Man et al. (2004). Protein Sci *13*, 240-254) of *OR11H7P* and its closest paralogs share a unique basic amino acid (histidine) in the 4th

transmembrane helix (Figure 3B). The observed conservation of this positively charged residue in *OR11H7P* orthologs of other mammals but not in numerous paralogs of the OR11H subfamily (Figure 3B) seems related to the binding of isovaleric acid, an odorant that bears a partial negative charge at neutral pH.

Functional expression of *OR11H7P*

A genotype-phenotype demonstrated a functional relation between *OR11H7P* and the odorant Isovaleric acid. To confirm the functional relationship, co-expression of OR11H7P with $G\alpha_s$-type G-protein and the Cystic fibrosis transmembrane regulator (CFTR) in *Xenopus* oocytes was undertaken and response to various concentrations of IVA was measured. The results of this experiment demonstrate that *OR11H7P* responds to 3mM of IVA (Figure 4). Although this odorant concentration is 3-fold higher than the concentration at threshold in individuals with hyperosmia to IVA, such differences are consistent with previous observations using this experimental system.

Inter-odorant threshold correlation

The ANOVA tests used for the genotype-phenotype associations (Figure 2) indicated that the genetic polymorphism in *OR11H7P* accounts for only ~20% of the variation in isovaleric acid thresholds indicating a contribution to individual threshold variation as a "general olfactory factor" that affects olfactory sensitivity to *all* odorants. Such inter-odorant threshold concordance within individuals has been as previously described. Figure S2 (supplementary data) demonstrates the inter-odorant threshold correlations for all odorant pairs (Figure S2 in the Supplementary Data). Repeating the phenotype-genotype association analysis using the average olfactory threshold score as a covariate did not affect the statistical significance of the results (See Figure 2). The effect of this "general olfactory factor" is further demarcated by the observation that the average threshold distribution is significantly broader than permuted one ($F= 1.83$, $P = 1.68X10^{-10}$, Figure 5A). The difference between the original and permuted values is considerably larger in the lower values of the average thresholds indicating that general hyperosmia is more prevalent than general hyposmia in the tested population. Figure 5B displays the variety of sensitivity phenotype combinations present. A significant gender contribution to

the average olfactory threshold variation (Figure 6) indicates a contribution of sex-related factors to the observed phenomena.

The observation that only people who carry at least one copy of the intact allele of OR11H7P display high sensitivity to the sweaty odorant IVA indicates a locus related to specific hyperosmia.

The observation of significant inter-odorant threshold correlations within individuals indicate that an inherent general olfactory factor contributes to the overall threshold variation in the human population.

Experimental Procedures

Subject recruitment

This study was approved by the Institutional Review Board (Helsinki committee) of the Meir hospital in Kfar Saba, Israel. Human subjects (unrelated, randomly selected individuals) were recruited in collaboration with the Israeli Blood Bank (Tel Hashomer) within their blood donation sessions. Subjects included 197 females and 246 males, aged 18-48, originating from three major Jewish ethnicities: 329 Ashkenazi, 61 Sephardic and 53 Ashkenazi- Sephardic admixtures). Every participant signed an informed consent form and filled a detailed questionnaire in order to exclude neurological impairments, nose injuries and other conditions with potential effect on human olfactory acuity. Age, gender, ethnic origin and smoking habits were also recorded.

Olfactory threshold measurements

Odorants, dissolved in light white mineral oil (Sigma) at 5 tenfold dilutions between 10^{-2}M and 10^{-6}M were presented in a Sniffin' Sticks kits (Hummel et al. (1997). Chem Senses *22*, 39-52). These were replaced every 3 months to reduce contaminations and odorant evaporation. The odorants used were Isoamyl acetate (98%, Aldrich, IAA), Isovaleric acid (99%, Sigma-Aldrich, IVA), L-Carvone (97%, Aldrich, LCA) and Cineole (Sigma-Aldrich, CIN). Detection thresholds were determined using an ascending staircase three-way forced choice procedure as previously described. Odor presentation by cap removal was for ~3 sec and presentation was ~2 cm in front both nostrils. A single failure led to the next higher concentration, and detection threshold was the concentration showing 4 successive correct identifications, assuring of a low

false positive rate (~0.01). To attain reduction of false negative detection, the immediate sub-threshold concentration was retested, and claimed threshold if successful. Inter-trial intervals were >20 sec and subjects were not given feedback on their performance during the test. All olfactory tests were conducted in well ventilated, temperature controlled odorless room. A second olfactory measurement was performed within 1-4 weeks after the first one for 82 randomly selected individuals to assess test-retest reproducibility.

SNP genotyping

Genomic DNA was extracted from 10 ml of peripheral blood using a DNA Isolation Kit for Mammalian Blood (Roche). The DNA concentrations were measured using the Genius spectrophotometer at 260nm (Tecan) and subsequently normalized to 2.5ng/μl. Aliquots of 2μl were distributed in 384 microtiter plates by the Biomek 2000 laboratory automation workstation (Beckman). SNP genotypes were assessed by the Matrix Assisted Laser Desorption/Ionization – Time-of-Flight (MALDI-TOF) mass spectrometry technology of Sequenom (Bray et al.(2001). Hum Mutat *17*, 296-304).This procedure involved multiplexes of 10-24 SNP reactions, automatically designed by the SpectroDESIGNER™ algorithm (Sequenom) and validated for specific genomic amplification using the UCSC In-Silico PCR (http://genome.ucsc.edu/). SNP genotyping was performed twice and inconsistencies were removed from further analyses. Overall, high genotyping efficiency (98% ± 3%) was achieved for the 52 polymorphic loci.

OR Functional expression

In-vitro functional expression of ORs were carried out as described according to Abaffy et al. (2006; J Neurochem *97*, 1506-1518.) . OR coding regions were amplified by PCR from human genomic DNA (BD Biosciences/Clontech, Palo Alto, CA, USA), subcloned into a pCI vector (Promega) containing the 20 amino acid N-terminal sequence of human rhodopsin (required for functional expression in this system) and confirmed by sequencing. The Stop codon (TAG) at position 679 of *OR11H7P* was corrected to a CAG codon for glutamine by QuickChange II site-directed mutagenesis kit (Stratagene, La Jolla, CA), and verified by sequencing. For functional recordings, 25 ng of the corresponding cRNA was injected into each *Xenopus laevis* oocyte, together with 10 ng Gαolf and 1 ng CFTR cRNAs, and response to the odorant IVA was measured by electrophysiological recording of the cAMP-iduced CFTR-dependent Cl^- current.

Results were measured as the ration of current amplitudes elicited by the odorant and the phosphodiesterase inhibitor IBMX. *Xenopus laevis* frogs were purchased from Nasco (Fort Atkinson, WI). The care and use of *X. laevis* frogs was approved by the University of Miami Animal Research Committee.

Figure legends

Figure 1

Olfactory threshold distributions.

Histograms for the measured olfactory thresholds of the four odorants, where concentrations are in molar (M) in the oil solution. Odorant were tested in the 377 genotyped individuals, except Cineole, which was tested in a randomly selected sub-sample of 200 subjects. A threshold score of 1 indicate individuals who couldn't detect the highest possible odorant concentration (10^{-2} M). Red, Yellow and Green respectively represent fractions out of the total sample for homozygote disrupted, heterozygote and homozygote intact. IAA, Isoamyl acetate; IVA, Isovaleric acid; LCA, L-Carvone; CIN, Cineole.

Figure 2

Genotype-phenotype association.

ANOVA *P*-values (computed by Matlab) for comparison between the threshold distributions of subjects belonging to the three genotype groups (Figure 1), using gender as a covariate. Segregating Pseudogene loci (SPGs) are enumerated as in Table 2 in the Supplementary Data. The broken line indicates the statistical significant value of $P = 0.05$ after Bonferroni correction for 172 tests. The two strongest *P*-values for IVA are for the genes marked in Figure 3. Significance remained also after using the "general olfactory effect" as an additional covariate (Black open circles in IVA panel). Odorants are marked as in Figure 1.

Figure 3

Genomic and structural analyses.

A. The genomic region associated with isovaleric hyperosmia. Arrow direction indicates gene genomic orientation; Red, green and yellow indicate pseudogenes, intact genes and segregating pseudogenes (SPGs) respectively. The two SPGs showing the strong association with isovaleric sensitivity are marked with single and double asterisks.

B. Alignment of 22 residues comprising the predicted odorant binding site of OR proteins. Amino acids are color coded according to their chemical characteristics. The *OR11H7P* differentiate from its closely related paralogs at positions 10 and 11 of the putative CDR.

Figure 4

The receptor *OR11H7P* responds to IVA.

A. Representative trace of the response of *Xenopus* oocyte expressing the OR11H7P receptor to 15 sec application of either IBMX or the odorant IVA at the concentrations indicated. B. Summary of the 6-9 recordings each from oocytes expressing *OR11H7P, OR52E4, OR8A1* and *OR12D2*. Responses were normalized to the 1mM IBMX response in the same oocyte.

Figure 5

Excess general hyperosmia.

A. Histograms of average olfactory thresholds (corrected for gender) are plotted. The significantly broader distribution of the original data (black) as compared to permutated data (dotted) (ANOVA F= 1.83, $P = 1.68X10^{-10}$) indicates excess of individuals with extreme threshold values, particularly in the hypersensitivity end of the distribution.

B. Combinations of odorant thresholds (corrected for gender) for the four odorants (marked as in Figure 1). Shown are hyperosmia (lowest 10% of thresholds in the entire sample, black), normosmia (middle 80%, grey) and hyposmia (highest 10%, white). Individuals with similar threshold patterns are clustered together. For clarity, only 50 of the total of 123 normosmic individuals are shown. The probability of observing 3 individuals defined as generally hyperosmic, having hyperosmia to all four odorants is computed as $\sim 10^{-12}$.

Figure 6

Inter-odorant threshold correlation.

Scatter plots for the original and shuffled thresholds of Isoamyl acetate (IAA) and Cineole (CIN). The significant correlation in panel A ($R = 0.52$, $P < 10^{-4}$) is lost in panel B ($R = 0.07$, $P = 0.36$). The significant gender difference (Table 1 in Supplementary Data) is also seen with females (Red) having lower thresholds than males (Blue).

Supplementary data

Figure S1

Test-retest reproducibility.

The 1st and 2nd thresholds (in $-\log_{10}$ M) of 82 individuals are plotted for the four odorants. Circle areas are proportional to the number of participants with similar scores. Test-retest reproducibility is demonstrated by the high correlation coefficients scores. In addition, the inter-individual threshold variance for all odorants ($Var_{tot} = 1.56 \pm 0.73$) was significantly higher (P=0.0049 ± 0.0005) than the intra-individual variance ($Var_{dif} = 0.78 \pm 0.35$), suggesting that inconsistencies of olfactory performance within individuals do not explain the entire observed threshold variance.

Figure S2

Inter-odorant threshold correlations.

The normalized thresholds (due to the gender effect) of individuals are plotted for all possible odorant pairs. The significant correlation coefficient scores ($0.02 \leq R \leq 0.5$) in all panels imply that a common denominator control the olfactory sensitivity towards all odorants.

	IAA	IVA	LCA	CIN
Gender	**0.0048**	**0.0050**	**0.0072**	**0.00003**
Age	0.5726	0.9880	0.5266	0.5245
Origin	0.1174	0.1371	0.4480	0.4474
Smoking	0.7156	0.7383	0.1681	0.7892

Suplementary Table 1

Olfactory threshold confounding factors.

P-values of ANOVA are given for the effect of Gender, Age, Ethnic origin, and Smoking on the olfactory thresholds of the different odorants. In Bold are the statistically significant *P*-values (<0.05).

No.	Gene information		Polymorphism information				Genotyping frequencies			Allele frequencies		Hardy-Weinberg equilibrium
	Name	Genomic location	Position	Type	Intact allele	Pseudogene allele	Homozygote Intact	Heterozygote	Homozygote pseudogene	Intact allele	Pseudogene allele	*P* of Chi square test
1	OR10X1	1@155.4	147	Stop	G	A	0.29	0.508	0.202	0.544	0.456	0.899
2	OR10J4P	1@156.2	593	Del(1)	T	-	0.867	0.13	0.003	0.932	0.068	0.838
3	OR2L8	1@244.6	650	Y226C	A	G	0.005	0.186	0.808	0.098	0.901	0.645
4	OR5H6	3@99.5	385	P137A	C	G	0.419	0.448	0.133	0.643	0.357	0.896
5	OR5H6	3@99.5	535	C187R	T	C	0.162	0.411	0.426	0.3675	0.6315	0.109
6	OR5AC1P	3@99.5	257	Del(1)	T	-	0.336	0.53	0.134	0.601	0.399	0.131
7	OR12D1P	6@29.4	556-572	Del(16)	GCCTGTGGGAACACTG	-	0.117	0.473	0.41	0.3535	0.6465	0.79
8	OR12D2	6@29.4	359	R130L	G	T	0.184	0.524	0.291	0.446	0.553	0.507
9	OR2J1P	6@29.4	574	Stop	C	T	0.296	0.494	0.209	0.543	0.456	0.998
10	# OR10C1	6@29.4	163	Stop	C	T	0.935	0.065	0	0.9675	0.0325	0.812
11	* OR10AC1P	7@142.7	267	In(1)	-	G	0.523	0.341	0.136	0.6935	0.3065	0.002
12	OR2F1	7@143.3	364	R130C	C	T	0.872	0.118	0.011	0.931	0.07	0.215
13	OR1B1	9@122.5	574	Stop	C	T	0.427	0.462	0.111	0.658	0.342	0.887
14	OR13C7P	9@35.9	435-436	In(2)	-	AA	0.034	0.247	0.719	0.1575	0.8425	0.376
15	OR8B4	11@123.6	532	C187R	T	C	0.52	0.379	0.101	0.7095	0.2905	0.302
16	OR8D2	11@123.6	365	R130H	G	A	0.592	0.366	0.042	0.775	0.225	0.647
17	OR8G1	11@123.6	777	Stop	C	G	0.227	0.539	0.235	0.4965	0.5045	0.325
18	OR4X2	11@48.4	81	Stop	C	G	0.807	0.19	0.003	0.902	0.098	0.323
19	OR51J1	11@5.0	299	C105Y	G	A	0.743	0.225	0.032	0.8555	0.1445	0.229
20	OR52B4	11@5.0	119	Del(1)	C	-	0.444	0.46	0.097	0.674	0.327	0.686
21	* OR51F1	11@5.0	274	Del(1)	C	-	0.422	0.408	0.17	0.626	0.374	0.046
22	OR52E1P	11@5.0	778	Del(1)	C	-	0.269	0.476	0.255	0.507	0.493	0.652
23	OR52Z1P	11@5.0	22	Del(1)	G	-	0.102	0.404	0.493	0.304	0.695	0.68
24	OR52R1	11@5.0	386	I134T	C	T	0.239	0.515	0.247	0.4965	0.5045	0.851
25	OR51B2	11@5.0	358	R130C	C	T	0.814	0.175	0.011	0.9015	0.0985	0.977
26	OR51G1	11@5.0	371	R130H	G	A	0.707	0.253	0.04	0.8335	0.1665	0.229
27	OR52H1	11@5.0	371	R130H	G	A	0.663	0.31	0.027	0.818	0.182	0.714
28	OR51Q1	11@5.0	706	Stop	C	T	0.285	0.495	0.221	0.5325	0.4685	0.992
29	OR52D1	11@5.0	662	Y226F	A	T	0.684	0.279	0.037	0.8235	0.1765	0.73
30	OR52L1	11@5.9	373	R130C	C	T	0.547	0.373	0.08	0.7335	0.2665	0.679
31	OR52N4	11@5.9	514	Stop	A	T	0.62	0.335	0.045	0.7875	0.2125	1
32	# OR5R1	11@55.6	362	D129R	A	G	0.928	0.067	0.005	0.9615	0.0385	0.137
33	OR5R1	11@55.6	364	R130C	C	T	0.597	0.357	0.046	0.7755	0.2245	0.89
34	OR5G3P	11@55.6	343	Del(1)	C	-	0.333	0.44	0.227	0.553	0.447	0.104
35	OR5AL1P	11@55.6	468-469	Del(2)	CT	-	0.851	0.146	0.003	0.924	0.076	0.694
36	OR5L1	11@55.6	858	P298S	C	T	0.006	0.133	0.861	0.0725	0.9275	0.978
37	OR8K3	11@55.6	365	R130L	G	T	0.363	0.461	0.176	0.5935	0.4065	0.695
38	# OR8J2P	11@55.6	190	Stop	C	T	0.008	0.08	0.912	0.048	0.952	0.054
39	OR5AR1	11@55.6	55	Stop	C	T	0.109	0.43	0.462	0.324	0.677	0.938
40	OR6Q1	11@57.7	685	Del(1)	C	-	0.719	0.26	0.021	0.849	0.151	0.97
41	OR1S1	11@57.7	365	R130H	G	A	0.214	0.466	0.32	0.447	0.553	0.547
42	OR10A6	11@7.8	860	P298L	C	T	0.597	0.345	0.058	0.7695	0.2305	0.856
43	* OR4K3P	14@19.5	622	Del(1)	C	-	0.608	0.378	0.014	0.797	0.203	0.006
44	OR4Q2P	14@19.5	531	In(1)	-	T	0.152	0.423	0.425	0.3635	0.6365	0.263
45	OR11H7P	14@19.5	679	Stop	C	T	0.308	0.475	0.217	0.5455	0.4545	0.708
46	OR4E1P	14@21.2	584-585	In(2)	-	AC	0.605	0.345	0.05	0.7775	0.2225	0.996
47	OR4E2	14@21.2	352	M126V	A	G	0.604	0.348	0.048	0.778	0.222	0.987
48	OR6J1	14@22.2	362	R130H	G	A	0.745	0.244	0.011	0.867	0.133	0.513
49	* OR1A1	17@3.1	853	P298S	C	T	0.308	0.435	0.257	0.5255	0.4745	0.046
50	# OR7C2	17@3.1	365	R130H	G	A	0.92	0.077	0.003	0.9585	0.0415	0.894
51	* OR3A1	17@3.1	374	R130Q	G	A	0.313	0.372	0.315	0.499	0.501	0
52	OR1P1P	17@3.1	523	Stop	A	T	0.014	0.252	0.734	0.14	0.86	0.651

Supplementary Table 2

OR SPGs information.

OR SPGs are ordered according to their genomic location. The four genes with minor allele frequency below 0.05 (Marked with #) and five genes with genotypes deviating significantly from Hardy-Weinberg ($P < 0.05$ of *Chi-square* test, marked with *) were not included in the phenotype-genotype analyses.

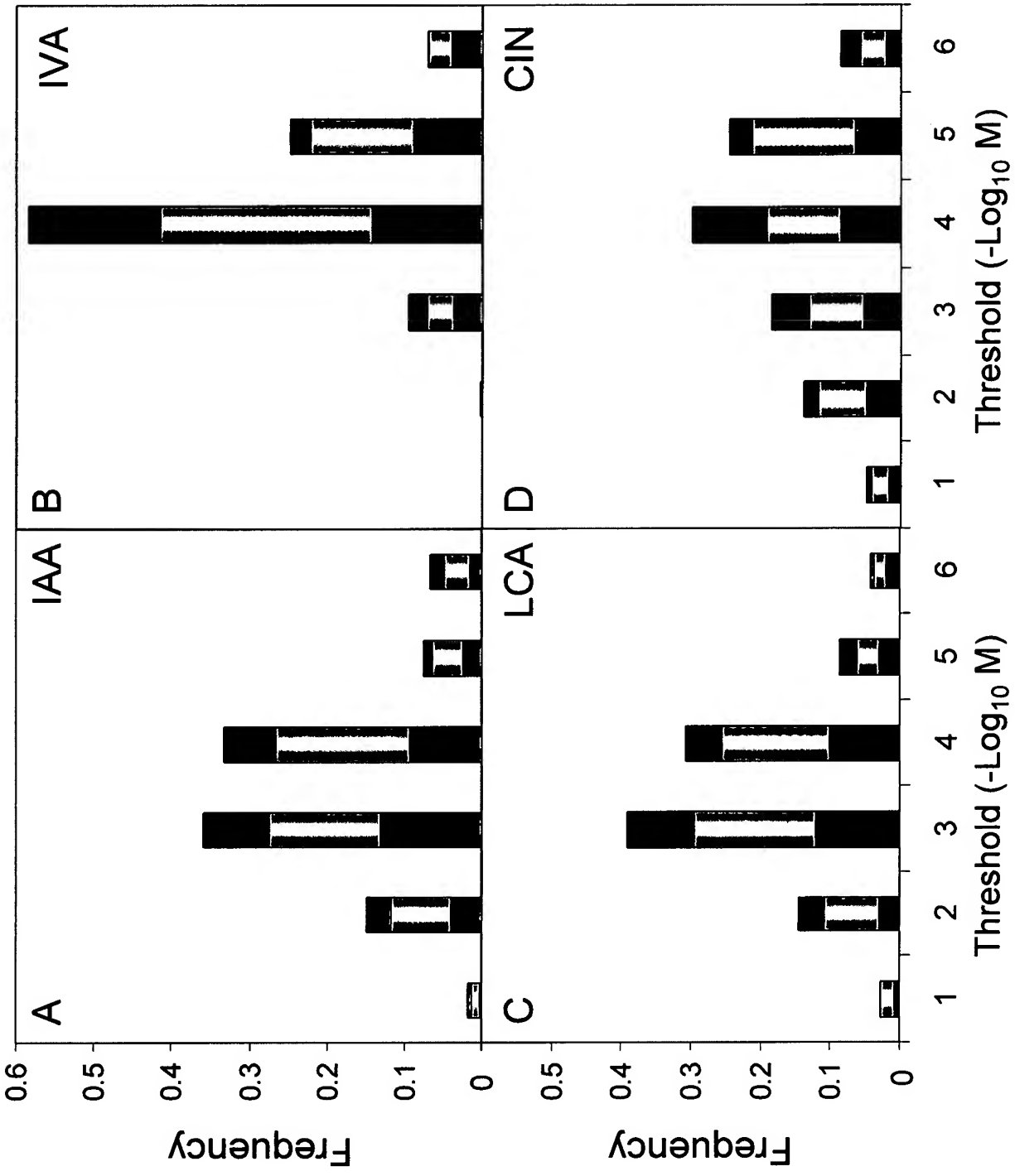


Figure 1

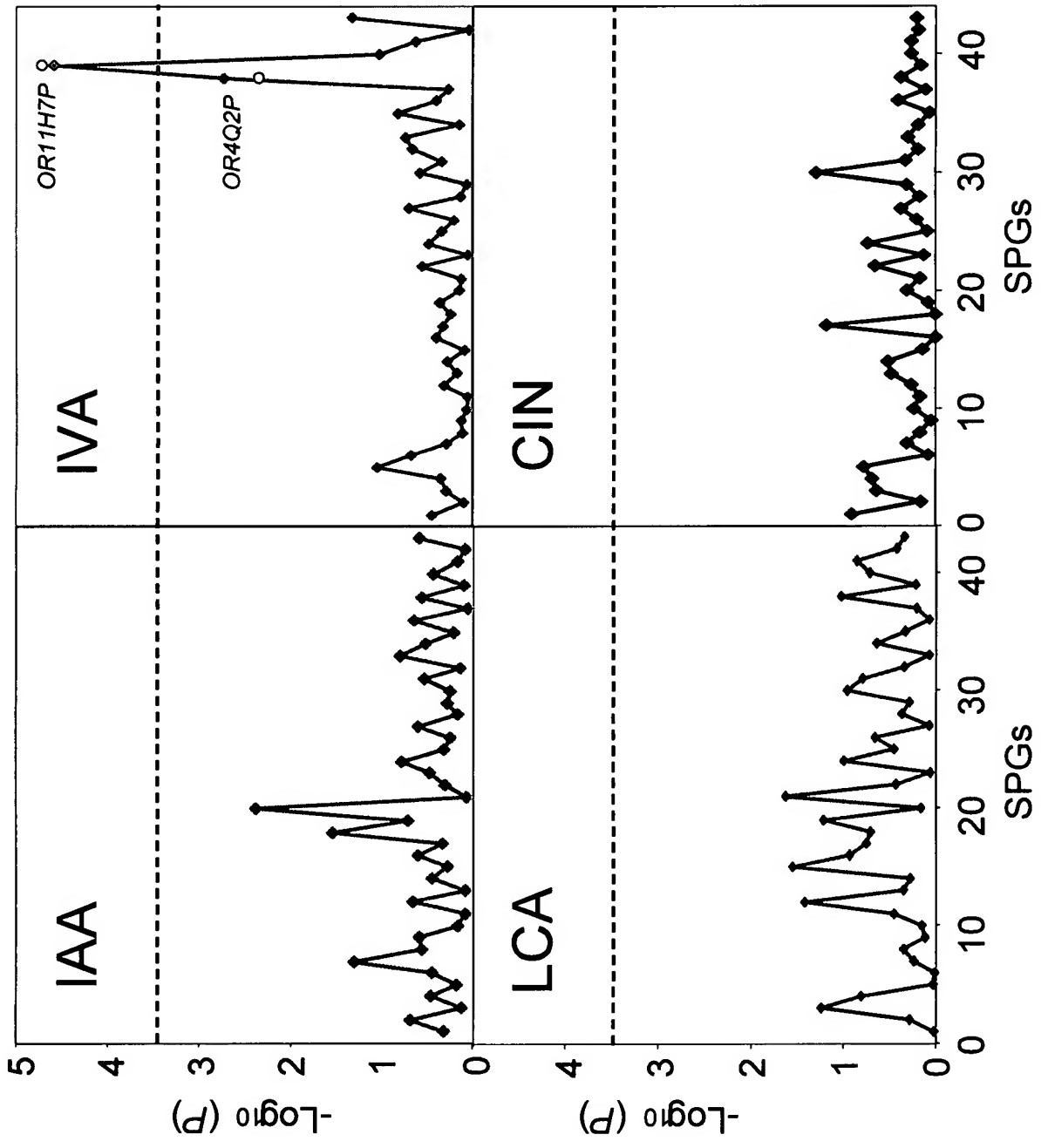
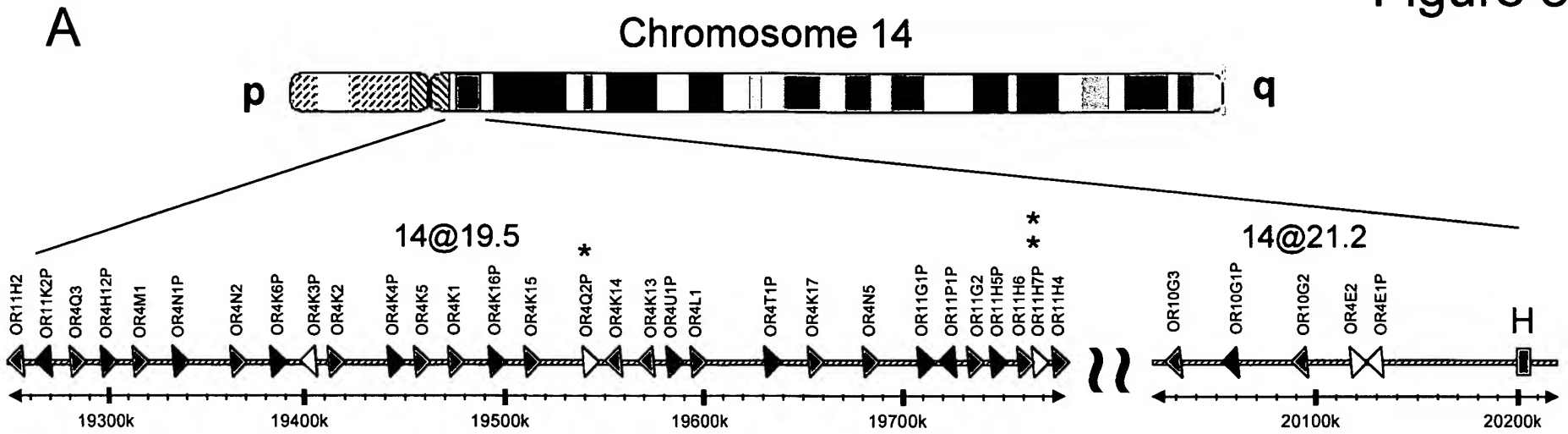


Figure 2

Figure 3

A



B

	OR / Position	TM2	TM3							TM4				EC2		TM5					TM6		TM7		
		1	2	3	4	5	6	7	8	9	10	11		12	13	14	15	16	17		18	19	20	21	22
Human	OR11H7P	Y	F	F	F	G	T		F	G	H	S		L	V	F	S	F	I		Y	I	T	L	S
	OR11H4	Y	F	F	F	G	T		L	G	Y	P		L	M	F	S	V	L		Y	V	T	L	S
	OR11H6	Y	F	F	F	G	T		F	G	Y	P		V	P		N	I	I		Y	L	T	L	T
	OR11H3P	Y	F	F	F	G	T		L	G	F	P		V	P		S	V	I		Y	L	T	L	A
	OR11H2	Y	F	F	F	G	T		L	G	F	P		V	P		S	V	I		Y	L	T	L	A
	OR11H13P	Y	F	F	F	G	T		L	G	F	P		V	P		S	V	I		Y	L	T	L	A
	OR11H12	Y	F	F	F	G	T		L	G	F	P		V	P		S	V	I		Y	L	T	L	A
	OR11H1	Y	F	F	F	G	T		L	G	F	P		V	P		S	V	I		Y	L	T	L	A
Mouse	mOR106-12	Y	F	F	F	G	T		F	G	H	P		L	V	F	S	F	I		Y	I	T	L	S
Rat	NG 003469	Stop	F	F	F	G	T		F	G	H	P		L	V	F	S	F	I		Y	I	T	L	S
Dog	cOR11H7P	Y	F	F	F	G	T		F	G	H	T		L	V	F	S	I	I		Y	I	T	L	S

Basic

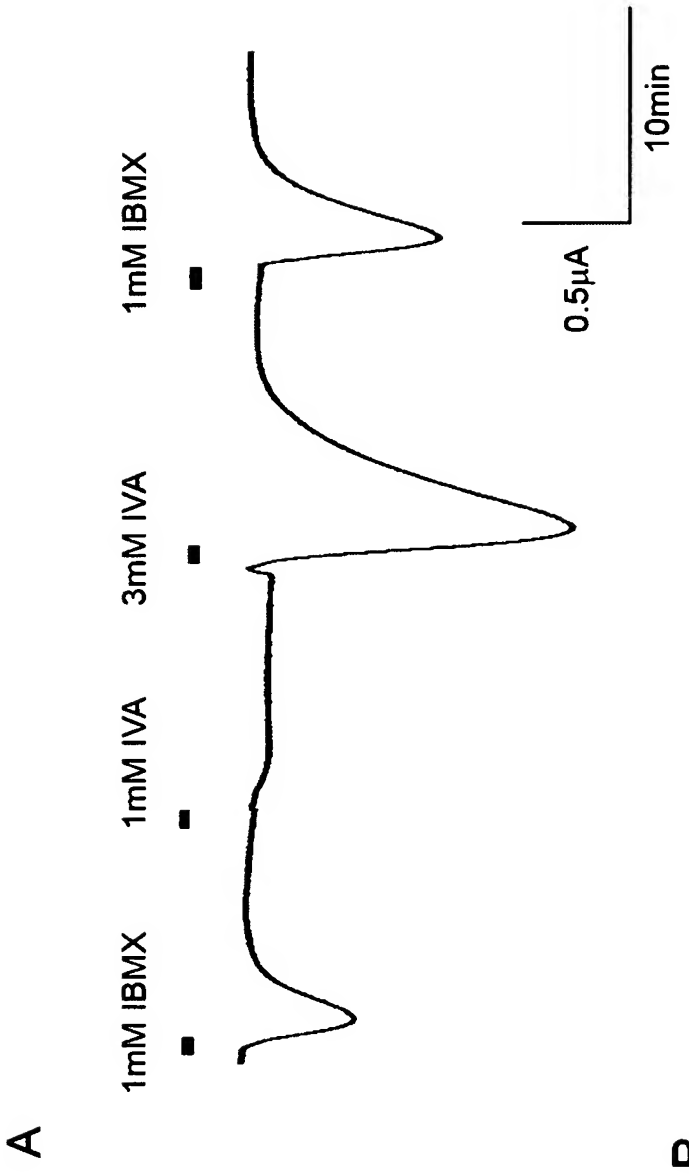
Hydrophobic, no charge

Aliphatic

Aromatic

Helix breaker

Cystein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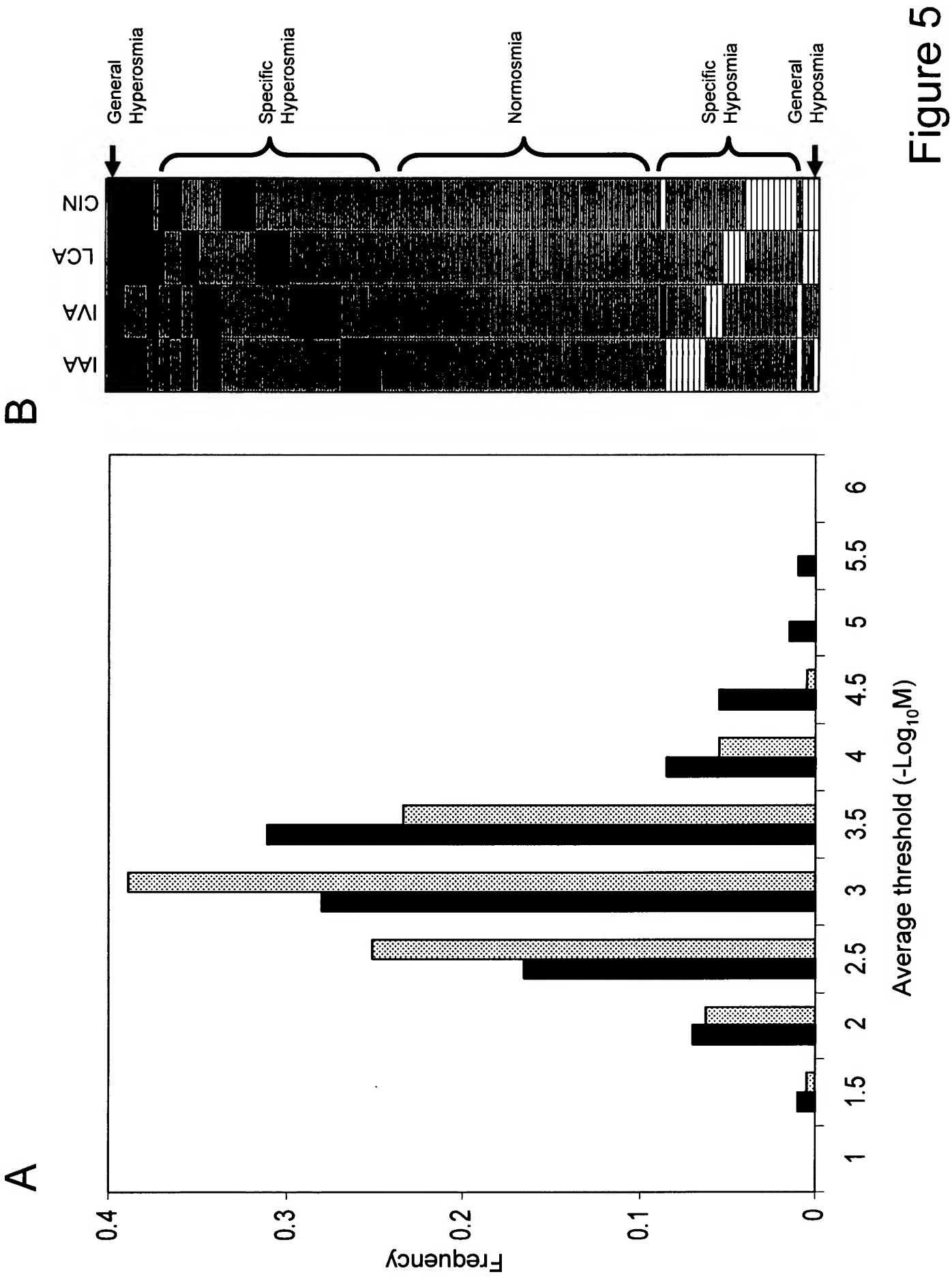
0.0
OR11H7P
OR52E4
OR8A1
OR12D2

Figure 4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5.5
6
Average threshold (-Log10M)
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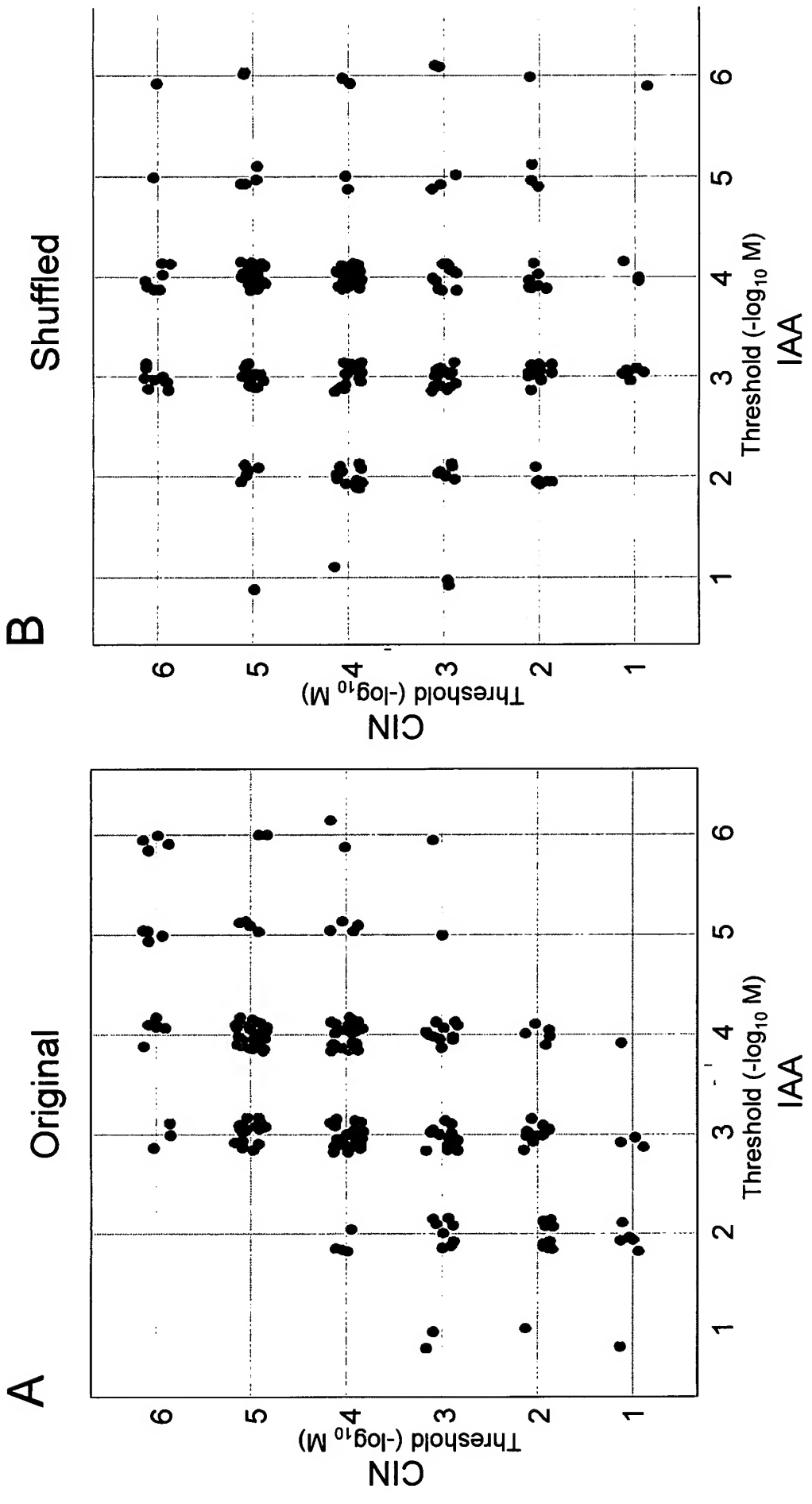
Specific Hyperosmia
Normosmia
Specific Hyposmia
General Hyposmia
CIN
LCA
IVA
IAA

Figure 5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4
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Figure 6

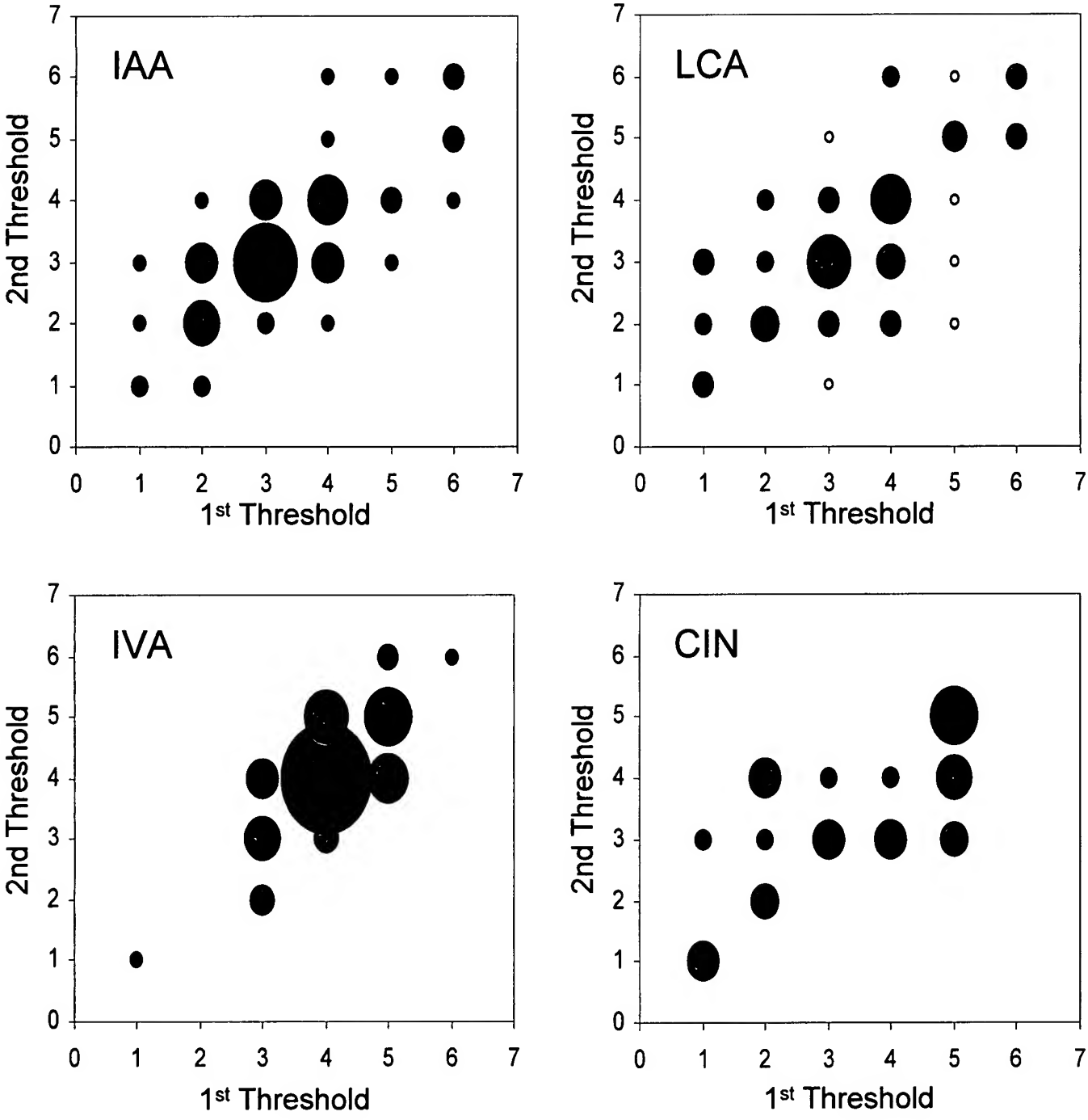

Figure S1

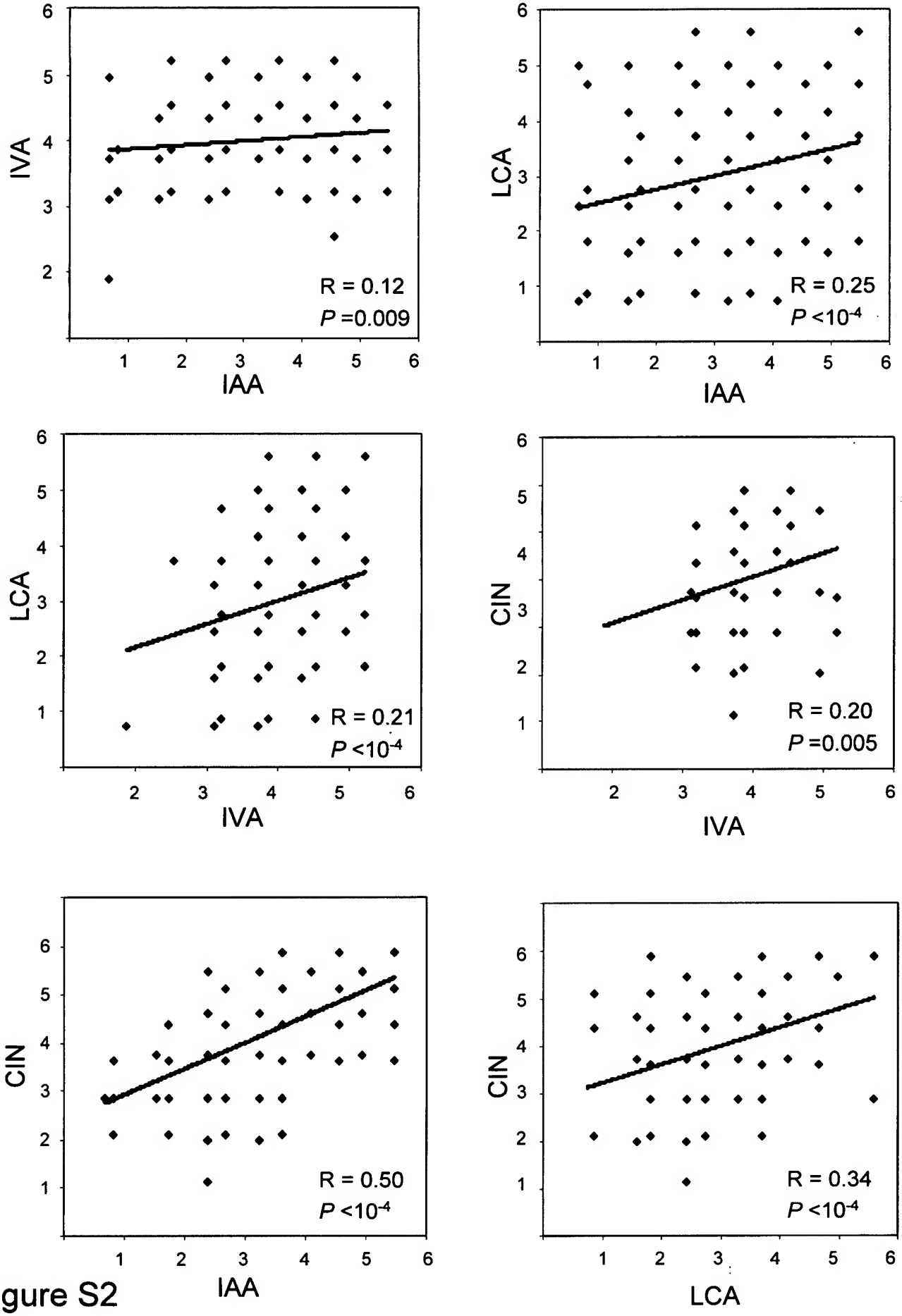
IVA
IAA
R = 0.12
P =0.009
LCA
IAA
R = 0.25
P <10-4
LCA
IVA
R = 0.21
P <10-4
CIN
IVA
R = 0.20
P =0.005
CIN
IAA
R = 0.50
P <10-4
CIN
LCA
R = 0.34
P <10-4

Figure S2

From: Shifra Ben-Dor [shifra.ben-dor@weizmann.ac.il]
Sent: Monday, 11 June, 2007 3:42 PM
To: Sinai Yarus
Subject: Re: inactive link

Hi,

There is a redirect from that link that seems to be broken.

The direct link is:

http://bip.weizmann.ac.il/HORDE/index.html

There is more information about the database in the About HORDE link:

http://bip.weizmann.ac.il/HORDE/aboutHORDE.html

If you have any questions about the database, the contact person is Dr. Tsviya Olender:

Tsviya Olender
Department of Molecular Genetics
Weizmann Institute of Science
Rehovot, Israel.
Tel: + 972-8-934-2572
tsviya.olender@weizmann.ac.il

Hope this helps,

shifra

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On Jun 11, 2007, at 5:05 PM, Sinai Yarus wrote:

> I encountered the following URL in a publication:
>
> http://bioinformatics.weizmann.ac.il/HORDE

Following the link produces a 404:Object not found error message.

Can you help me ascertain what was in HORDE

Very truly yours,

Dr. Sinai Yarus, Patent Attorney
EHRLICH & FENSTER
G. E. Ehrlich (1995) Ltd.
Ayalon Tower, 15th Floor
11 Menachem Begin Street
52 521 Ramat Gan, Israel
Tel.: 03-6127676 Fax.: 03-6127575

My address: sinai@ipatent.co.il
Our Website: www.ipatent.co.il

results support the scope of the claims as they are currently before the Examiner. In addition, Supplementary figure 2 suggests that sensitivity to isovaleric acid is generally indicative of olfactory sensitivity.

As an Olfactory Receptor Researcher, it is my opinion that one of ordinary skill in the art would intuitively understand the utility of ***typing the subject with regard to the subject's olfactory perception***. Examples of cases where it can be advantageous to screen individuals for olfactory perception include, but are not limited to *a priori* screening for employment positions in olfaction related professions (e.g. sommelier, whiskey blender, perfume formulary). I point out that a molecular assay for olfactory acuity mitigates to a great degree interfering factors (e.g. temporary illness) which can interfere with performance based assays of olfaction. With respect, I assert that this constitutes a specific and substantial utility.

Results presented in Appendix A clearly demonstrate that the claimed invention has utility according to 35 USC § 101. Additionally, results presented in Appendix A clearly demonstrate that the written description/enablement of the specification as originally filed meet the requirements of 35 USC § 112. As a result, rejections of claims 23-26, 28 and 29 under 35 USC § 101 and/or 35 USC § 112 seems unfounded.

My signature below confirms the authenticity of the results, the fact that the results were achieved using methods described in the disclosure and that the results provide the requisite degree of enablement for what is claimed.

I understand that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United states Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

July 24, 2007

Doron Lancet

Dr. Doron Lancet
The Weizmann Institute of Science
Rehovot, Israel